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A SUBSTANTIAL NUMBER OF ASYMMETRIC AXOSOMATIC SYNAPSES IS A CHARACTERISTIC OF THE GRANULE CELLS OF THE HIPPOCAMPAL DENTATE GYRUS

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The types of axosomatic synapses for pyramidal neurons of the Ammon's horn and granule cells of the dentate gyrus were investigated in the rat hippocampus. Local circuit neurons in both of these regions were also included. The mean number of axosomatic synapses was higher for pyramidal neurons than granule cells. Practically all of these synaptic contacts were symmetric for the pyramidal neurons of the CA1 area, whereas the percentage of asymmetric axosomatic synapses for granule cells and local circuit neurons was 21% and 26%, respectively. This finding is interesting because granule cells appear to be the only known projection neuron type to possess such a high percentage of asymmetric axosomatic synapses.

It is generally accepted that the somata of neocortical pyramidal neurons are contacted by terminals that form exclusively symmetric synapses [2, 8–10, 19] and arise mainly from GABAergic local circuit neurons [5, 13, 15]. In contrast, the somata of GABAergic neurons in neocortex are contacted by terminals that form both symmetric and asymmetric synapses [5, 11, 13]. Unfortunately, this rule concerning axosomatic synapses may not be strictly applied because a class of non-GABAergic neurons that could be either projection or local circuit neurons displays both types of axosomatic synapses [21].

We wanted to know if hippocampal projection neurons were similar to neocortical ones in this respect. A few studies have indicated that both pyramidal and granule cells in the hippocampal region may occasionally form axosomatic synapses that are not symmetric. Kosaka [7] reported the presence of transitional types of synapses on the pyramidal cell bodies and axon initial segments in the CA3 region. In addition, some granule cells in the dentate gyrus were shown to be contacted by degenerated commissural terminals that formed asymmetric axosomatic synapses

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[18]. Recent reports suggest that two other afferent pathways display terminals that form asymmetric synapses with granule cell bodies [1, 3]. Furthermore, asymmetric axosomatic synapses have been reported for newly developed granule cells [6]. These results suggest a basic difference between neocortical and hippocampal projection neurons. The present study was undertaken to determine the frequency of asymmetric axosomatic synapses for two types of hippocampal projection neuron, pyramidal and granule cells. In addition, local circuit neurons from both of these regions of the hippocampal formation were included in this analysis.

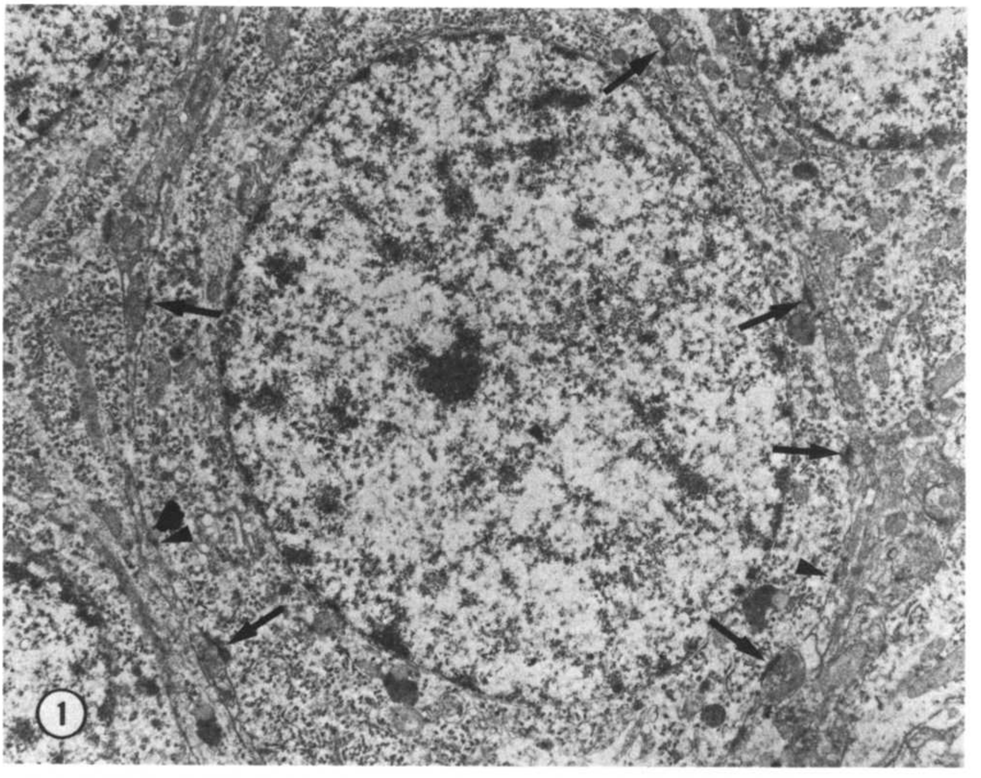
Ten adult albino rats (Wistar strain) were used in these experiments. The animals were perfused with a solution containing 4.0% paraformaldehyde–1.25% glutaraldehyde–0.002% calcium chloride in a 0.12 M phosphate buffer, pH 7.2. The perfused animals were kept overnight in the refrigerator before dissecting out the hippocampus the following day. The hippocampi were processed for electron microscopy using a routine schedule that included post-staining with 2% OsO_4 and embedded in Epon. Serial thin-sections were taken from blocks that contained the CA1 area and dentate gyrus of the hippocampus. Slot grids (1×2 mm) were used because they facilitated the examination of large areas. Thus, 80–100 pyramidal neurons of the CA1 area or 100–150 granule cells of the dentate gyrus could theoretically be evaluated in a single thin-section. The sections were stained with uranyl acetate and lead citrate and examined with a Philips 300 electron microscope. Only areas that displayed good preservation and an adequate section thickness for clear identification of synapses were chosen for this analysis. A total of 823 axosomatic synapses that contacted 313 neurons was analysed in this study.

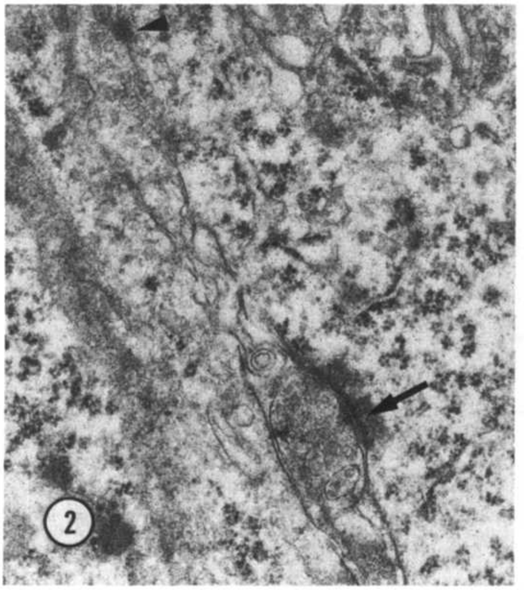
In electron microscopic preparations of the granule cell layer of the dentate gyrus, the most common neuron was the granule cell, although somata of basket cells were also found in this layer [14, 16]. The somata of granule cells were contacted by terminals that formed both symmetric and asymmetric synapses (Fig. 1). The mean number of axosomatic synapses for all granule cells per thin-section was about 2.2, and those granule cells at the hilar border had slightly more synapses than the ones that border with the molecular layer (Table I). The most surprising finding was the

TABLE I

AXOSOMATIC SYNAPSES IN THE DENTATE GYRUS AND HIPPOCAMPUS

Cell type	Number of somata	Total number of axosomatic synapses (mean ± S.D.)	Number of asymmetric synapses (% of total)
Granule cells at the hilar border	125	299 (2.39 ± 1.35)	71 (23.75%)
Granule cells at the border with the molecular layer	80	160 (2.00 ± 1.17)	30 (18.75%)
CA1 pyramidal cells	68	234 (3.44 ± 0.92)	–
Local circuit neurons	40	130 (3.25 ± 1.22)	34 (26.15%)
Total	313	823	135





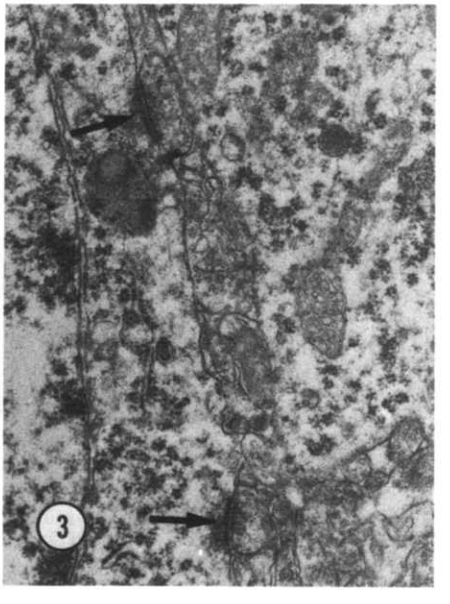


Fig. 1. Electron micrograph of a granule cell which displays 8 axosomatic synapses in one thin-section. Six of them are asymmetric (arrows) and two are symmetric (arrowheads). $\times 8000$.

Fig. 2. Enlargement of two of the axosomatic synapses on the left side of the soma in Fig. 1. One is asymmetric (arrow) and the other is symmetric (arrowhead). $\times 23{,}000$.

Fig. 3. Enlargement of two asymmetric axosomatic synapses (arrows) from the right side of the granule cell in Fig. 1. $\times 20{,}000$.

large number of asymmetric axosomatic synapses. The granule cells at the hilar border displayed a higher frequency of this synaptic type than those at the border with the molecular layer. In the illustrated example of a granule cell with the highest number of axosomatic synapses, 5 of 8 synapses were asymmetric (Figs. 1-3). On an average, 21% of the axosomatic synapses were asymmetric and in 4 cases, the same terminal formed asymmetric contacts with two adjacent granule cells (Fig. 4).

The pyramidal neurons from the CA1 area were examined and they had mainly symmetric axosomatic synapses. However, some of the axosomatic contacts were not clearly symmetric because they resembled the intermediate type of synapse described by Kosaka [7]. Yet, none of the synapses with pyramidal somata were asymmetric. The mean number of axosomatic synapses with pyramidal neurons was 3.4 per thin-section (Table I).

In both the dentate gyrus and the CA1 area, local circuit neurons were identified using established criteria [4, 14, 16, 20]. The somata of these neurons displayed both asymmetric and symmetric axosomatic synapses as described previously. The mean number of axosomatic synapses per thin-section was 3.25 for local circuit neurons and 26% of the total were asymmetric (Table I).

This study shows that asymmetric axosomatic synapses with granule cells are not occasional occurrences but are quite common (about one in 5). Therefore, a substantial number of asymmetric axosomatic synapses is a characteristic for the granule cells of the hippocampal dentate gyrus. In contrast, the pyramidal neurons of the Ammon's horn have, for the most part, only symmetric axosomatic synapses.

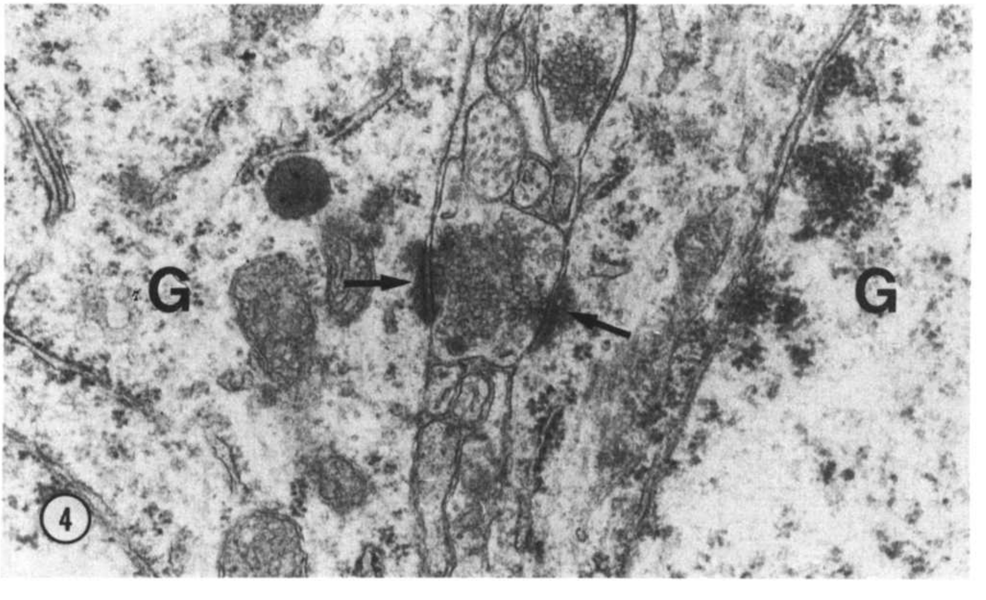


Fig. 4. Electron micrograph of an axon terminal that forms asymmetric synapses (arrows) with two neighboring granule cells (G) in the same section. ×25,000.

This morphological difference between pyramidal and granule cells may provide an anatomical substrate for some observed functional differences. Recent electrophysiological data suggest that pyramidal neurons of the CA1 area are silent during the synchronized theta activity while the granule cells are active [12, 17]. The substantial number of asymmetric (excitatory type) axosomatic synapses for granule cells may provide a basis for this difference in activity between granule and pyramidal cells.

It is interesting to note that the percentage of axosomatic synapses that are asymmetric for granule cells is almost equal to that found for the local circuit neurons in the hippocampal formation. These findings suggest that the granule cells of the dentate gyrus have a feature that is typical for local circuit neurons but not for projection neurons of the cerebral cortex.

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